
□ 1: Am J Pathol. 1988 May;131(2):183-9.

A simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues.

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Routine fixation and paraffin embedding destroys many hematopoietic and lymphoid differentiation antigens detected by flow cytometry or frozen section immunohistochemistry. On the other hand, morphologic evaluation is difficult in flow cytometric or frozen section studies. A simplified three-step plastic embedding system using acetone-fixed tissues embedded in glycol-methacrylate (GMA) resin has been found to provide both excellent morphologic and antigenic preservation. With our system, a wide variety of antigens are detected in plastic sections without trypsinization or prolonged embedding procedures; pan-B (CD19, CD22), pan-T (CD7, CD5, CD3, CD2), T-subset (CD4, CD8, CD1, CD25) markers as well as surface immunoglobulin and markers for myeloid and mononuclear-phagocyte cells are preserved. In summary, modifications of plastic embedding techniques used in this study simplify the procedure, apparently achieve excellent antigenic preservation, and facilitate evaluation of morphologic details in relation to immunocytochemical markers.

PMID: 3282442 [PubMed - indexed for MEDLINE]

☐ 1: Immunol Ser. 1990;53:339-56.

Prostate cancer-associated markers.

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Immunodiagnosis of prostate cancer is at a more advanced stage than that of most other tumors. Two well-known markers, prostatic acid phosphatase and prostate-specific antigen, have been used in the clinical management of patients. Prostate-specific antigen is a more sensitive and reliable marker than prostatic acid phosphatase. Serum prostate-specific antigen is effective in monitoring disease status, predicting recurrence, and detecting residual disease. Prostate-specific antigen is a tool for the histological differential diagnosis of metastatic carcinomas, especially in the identification of metastatic prostate tumor cells in distant organs and in the differentiation of primary prostate carcinoma from poorly differentiated transitional cell carcinoma of the bladder. Few data on biological function are available. Prostatic acid phosphatase functions as a phosphotyrosyl-protein phosphatase and prostate-specific antigen as a protease. Physiological function in the prostate remains to be elucidated. Several of the prostate-specific and prostate-tumor-associated antigens, as well as a putative prostate tumor-specific antigen, as recognized by monoclonal antibodies are available. Clinical evaluation of these potential markers is not yet available.

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□ 1: Clin Chem. 2000 Jul;46(7):896-900.

Prostate-specific antigen: a cancer fighter and a valuable messenger?

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BACKGROUND: Prostate-specific antigen (PSA) is a valuable prostatic cancer biomarker that is now widely used for population screening, diagnosis, and monitoring of patients with prostate cancer. Despite the voluminous literature on this biomarker, relatively few reports have addressed the issue of its physiological function and its connection to the pathogenesis and progression of prostate and other cancers.

APPROACH: I here review literature dealing with PSA physiology and pathobiology and discuss reports that either suggest that PSA is a beneficial molecule with tumor suppressor activity or that PSA has deleterious effects in prostate, breast, and possibly other cancers.

CONTENT: The present scientific literature on PSA physiology and pathobiology is confusing. A group of reports have suggested that PSA may act as a tumor suppressor, a negative regulator of cell growth, and an apoptotic molecule, whereas others suggest that PSA may, through its chymotrypsin-like activity, promote tumor progression and metastasis. **SUMMARY:** The physiological function of PSA is still not well understood. Because PSA is just one member of the human kallikrein gene family, it is possible that its biological functions are related to the activity of other related kallikreins. Only when the physiological functions of PSA and other kallikreins are elucidated will we be able to explain the currently apparently conflicting experimental data.

PMID: 10894830 [PubMed - indexed for MEDLINE]

□ 1: Cytogenet Cell Genet. 2000;90(1-2):93-101.

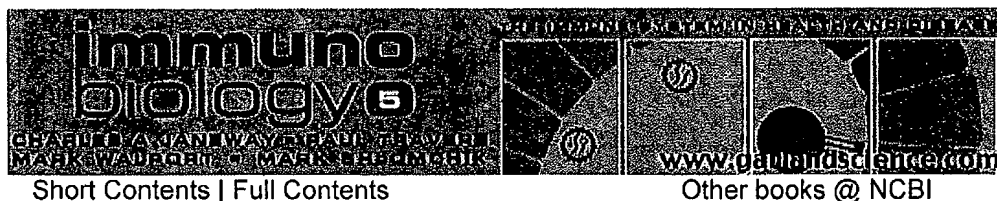
DNA methylation and chromosome instability in lymphoblastoid cell lines.

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In order to gain more insight into the relationships between DNA methylation and genome stability, chromosomal and molecular evolutions of four Epstein-Barr virus-transformed human lymphoblastoid cell lines were followed in culture for more than 2 yr. The four cell lines underwent early, strong overall demethylation of the genome. The classical satellite-rich, heterochromatic, juxtacentromeric regions of chromosomes 1, 9, and 16 and the distal part of the long arm of the Y chromosome displayed specific behavior with time in culture. In two cell lines, they underwent a strong demethylation, involving successively chromosomes Y, 9, 16, and 1, whereas in the two other cell lines, they remained heavily methylated. For classical satellite 2-rich heterochromatic regions of chromosomes 1 and 16, a direct relationship could be established between their demethylation, their undercondensation at metaphase, and their involvement in non-clonal rearrangements. Unstable sites distributed along the whole chromosomes were found only when the heterochromatic regions of chromosomes 1 and 16 were unstable. The classical satellite 3-rich heterochromatic region of chromosomes 9 and Y, despite their strong demethylation, remained condensed and stable. Genome demethylation and chromosome instability could not be related to variations in mRNA amounts of the DNA methyltransferases DNMT1, DNMT3A, and DNMT3B and DNA demethylase. These data suggest that the influence of DNA demethylation on chromosome stability is modulated by a sequence-specific chromatin structure. Copyright 2000 S. Karger AG, Basel.

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Navigation

About this book

Appendix I. Immunologists' Toolbox

Immunization.
The detection, measurement, and characterization of antibodies and their use as research and diagnostic tools.

➔ Isolation of lymphocytes.
Characterization of lymphocyte specificity, frequency, and function.
Detection of immunity *in vivo*.
Manipulation of the immune system.

Immunobiology ➔ Appendix I. Immunologists' Toolbox ➔
Isolation of lymphocytes.

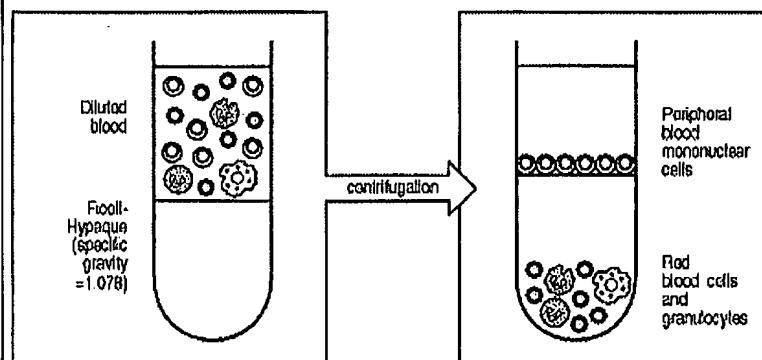


Figure A.23. Peripheral blood mononuclear cells can be isolated from whole blood by Ficoll-Hypaque™ centrifugation. Diluted anticoagulated blood (left panel) is layered over Ficoll-Hypaque™ and centrifuged. Red blood cells and polymorphonuclear leukocytes or granulocytes are more dense and centrifuge through the Ficoll-Hypaque™, while mononuclear cells consisting of lymphocytes together with some monocytes band over it and can be recovered at the interface (right panel).

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☐ PubMed

PROGRAM DESCRIPTION

Centre d'Etude du Polymorphisme Humain (CEPH): Collaborative Genetic Mapping of the Human Genome

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Elsewhere in this issue of *Genomics* is the first consortium map, that of chromosome 10, from the CEPH collaboration to map the human genome genetically. The map is truly a collaborative achievement, in that the underlying genotypes represent the efforts of laboratories collaborating with each other and with CEPH to produce a primary genetic map of the genome, consisting of polymorphic markers placed at approximately 20-cM intervals along each of the human autosomes and the X chromosome. Such a map provides a tool for the systematic localization of genes that determine inherited diseases and of other genes of interest. Genetic localization can be the first step in the development of diagnostic tests and isolation of a disease-determining gene. The purpose of this program description is to provide information about CEPH and the basis and nature of the collaboration.

CEPH

The Centre d'Etude du Polymorphisme Humain (CEPH)² is a nonprofit research institute that makes available to the scientific community a valuable research resource. CEPH is committed to (1) make available to the scientific community DNA samples from a panel of reference families for the determination of genotypes for various DNA polymorphisms which may be used for the construction of the genetic map of the human genome and for other research areas dependent on access to such a common set of families, and (2) provide to the contributors of genotypes a compilation of all data that accumulate on the panel of families.

CEPH Collaboration

The CEPH collaboration to map the human genome was organized in 1984 to hasten construction of a primary human

genetic map with DNA polymorphisms (Botstein *et al.*, 1980). A key premise of the CEPH collaboration is that the human genetic map will be efficiently achieved by collaborative research on DNA from the same sample of families. To this end, CEPH provides to collaborating investigators high-quality cellular DNA produced from cultured lymphoblastoid cell lines (LCL) derived from each member of a reference panel of large nuclear families/pedigrees and a database contributed to and shared by these investigators. Collaborating investigators determine genotypes with their probes and the DNA from the CEPH panel to test the families for segregation of these genetic markers. They then contribute the genotypes to CEPH for preparation of a database which is returned to them for linkage analysis and map construction. As of October 1, 1989, 63 research laboratories in the United States (36), Canada (2), Europe (20), South Africa (2), Japan (2), and Australia (1) collaborate with CEPH in this manner.

CEPH Reference Family Panel

Families with large sibships, living parents, and grandparents are especially informative for linkage mapping (White *et al.*, 1985). From 100 families available from various sources, selected not for disease but for large sibship size, an initial group of 40 families was defined for the CEPH reference panel by the original group of collaborating investigators. Table 1 shows the geographic origins of these families and the contributors of the LCLs to CEPH. These are Caucasian families. The mean sibship size for these 40 families, based on those individuals for whom there are LCLs, is 8.3; no family has less than 6 offspring, and 23 families have 8 or more offspring. LCLs are available for all 4 grandparents in each of 29 families of the reference panel.

LCLs of the reference panel are stored in liquid nitrogen at three geographically separate repositories: Paris and Lyon, France; and Salt Lake City, Utah. These LCLs are not distributed by CEPH within or outside of the collaboration. They are used only as a source of DNA for the collaboration. LCLs for 11 of the panel families are available from the NIGMS Human Genetic Mutant Cell Repository located at the Coriell Institute for Medical Research in Camden, New Jersey.

Approximately 20 mg of DNA is prepared, as needed, from each LCL, and aliquots of 200 or 400 μ g are distributed to each collaborating investigator. There is no charge to collaborating investigators for the DNA. The DNA is prepared by classical methods, scaled up to preparation of milligram amounts, which include lysis with proteinase K and SDS, extraction with phenol, and precipitation with isopropanol. The quality of each preparation of CEPH DNA is routinely controlled by testing for concentration, molecular size, digestibility with two different restriction enzymes, and contamination with vector sequences (Southern blot hybridized with a cosmid vector). In addition, each DNA preparation is hybridized with at least two highly variable ("minisatellite") probes (Wong *et al.*, 1987) to confirm identity of the LCL source and to detect contamination with DNA from another individual.

¹ All authors are members of the CEPH Executive Committee.

² Founded in 1983 by J.D. and D.C.

SPECIAL FEATURE

TABLE 1
Sources of CEPH Family Panel

Utah	27 families ^a	R. White
France	10 families	J. Dausset
Venezuela	2 families ^b	J. Gusella
Pennsylvania (Old Order Amish)	1 family ^c	J. Egeland

^a LCLs from 9 families available from NIGMS Human Genetic Mutant Cell Repository, Camden, NJ.

^b LCLs from 1 of 2 families available from NIGMS Human Genetic Mutant Cell Repository.

^c LCLs available from NIGMS Human Genetic Mutant Cell Repository.

DNA Polymorphisms

The family panel DNA is being tested for at least 1200 DNA polymorphisms. The probes being used within the collaboration detect polymorphism within restriction enzyme sites of low copy number or unique DNA segments, due to varying numbers of tandemly repeated, relatively small sequences, or VNTRs (Nakamura *et al.*, 1987), and of centromere-associated alpha satellite DNA (Willard *et al.*, 1986). Some collaborating investigators have begun to use genetic markers with family panel DNA that are based on detection of length variations of very small sequences amplified by PCR (Weber and May, 1989; Litt and Luty, 1989). Phenotypes for classical, serological, and electrophoretic polymorphic markers are also available for the family panel.

CEPH Database

CEPH collaborating investigators have agreed on two basic rules concerning their participation within the collaboration: (1) A CEPH collaborating investigator is committed to screening the reference panel by determining genotypes for all 40 parent pairs with each probe and enzyme combination used and then following segregation in each informative family (at least one heterozygous parent) in his or her own laboratory or collaboratively. (2) Collaborating investigators are committed to sending genotypic data to CEPH for inclusion in the CEPH database no later than publication of the data themselves or of an article based on the data.

Genotypic data generated from family panel DNA are processed into uniform format by a set of programs developed at CEPH (J.-M.L.) for use with IBM PCs or compatible models and sent to all collaborating investigators. The data files thus prepared are sent to CEPH to be merged into the CEPH database.

There are two components of the database: The CEPH collaborative database is available only within the collaboration. Two classifications of data are recognized in the collaborative database, *unpublished* and *published*. The first are those data that have not been used for a publication. These are privileged data, requiring permission from the contributor for use (e.g., publication) by another collaborating investi-

gator. Published data are automatically available for inclusion in CEPH consortium maps (see below) and after a lag period will be released to the CEPH public database. After 2 years in the collaborative database, unpublished data become published data. The CEPH public database, currently being organized, will contain published data released from the collaborative database. Data in the public database will be available to the general scientific community.

Table 2 summarizes the contents of the CEPH collaborative database. As of July 1989, the database contains genotypic data for 1061 genetic markers localized to all the autosomes and the X chromosome (including the pseudoautosomal region). Approximately 20% of these marker systems have four or more alleles.

New CEPH Activities

As the primary map of the human genome nears completion, there is growing interest in a higher resolution genetic map, perhaps of the order of 1–2 cM. Availability of a higher resolution map will increase the efficiency and precision of localization of genes. In order to support construction of a high-resolution map of the genome, CEPH is in the process of increasing the family panel to 61 large nuclear families/pedigrees. LCLs from 21 additional families have already been received from Utah, and stocks of DNA are being prepared from them. The mean sibship size for the total of 61 families in the augmented panel is 8.5, and LCLs for all 4 grandparents are available for each of 44 families. These 21 families provide an additional advantage for genetic mapping in that they have already been genotyped for approximately 500 genetic markers used in the CEPH collaboration.

CEPH has undertaken a project to enhance the use of the primary map of the genome in localizing genes of interest. The idea is to collect and produce quantities of ready-to-label probes for the mapped primary markers and distribute these to biomedical scientists who wish to localize disease-determining genes and other genes of interest. Probes to be distributed will be those for markers chosen from CEPH consortium maps based on criteria that include heterozygote frequency and position on the genetic map of a chromosome. Collections of probes ("kits") will be available for each chromosome. By using the probes in a kit to test a large kindred or group of families in which a genetic disease with the appropriate chromosome assignment is segregating, an inves-

TABLE 2
CEPH Database

Version	No. of contributing labs	No. of markers	No. of markers with 4 or more alleles	Date
V1	17	171	28	April 1987
V2	29	744	143	March 1988
V3	33	1061	204	July 1989

tigator should be able to find linkage to one, two, and perhaps more markers if a sufficient number of informative meioses are available. Again, if data from a sufficient number of meioses are available, we would expect that a gene of interest without a chromosome assignment could be localized with markers from all of the kits. Investigators using these kits for primary mapped markers will be invited to contribute the genotypes they determine from families being tested with the probes to a database provided by CEPH. This project, being carried out in collaboration with the American Type Culture Collection, is sponsored by the National Institutes of Health.

Progress toward the Primary Genetic Map of the Human Genome

The number of markers for which there are genotypic data in the CEPH database suggests that the primary human genetic map is nearing completion. Partial or nearly complete³ primary linkage maps based on genotypes determined from reference panel DNA with probes from a single or small group of CEPH collaborating laboratories have been published for many of the chromosomes (see, for example, Donis-Keller *et al.*, 1987; O'Connell *et al.*, 1989; Lathrop *et al.*, 1988; Nakamura *et al.*, 1988; Warren *et al.*, 1989). CEPH consortium maps, the first of which appears in this issue of *Genomics*, will provide reference primary linkage maps of each chromosome based on the combined genotypic data within the collaboration.

CEPH Consortium Maps

The primary genetic map of the human genome, based on all genotypes determined from the CEPH families, will be communicated to the scientific community through a series of consortium maps, one for each chromosome. These maps will represent the ultimate validation of genotypic data in the CEPH database. Each consortium map will be prepared by a committee of collaborating investigators who have contributed genotypic data for markers localized to the particular chromosome. A chromosome-specific database, containing genotypes for all relevant markers, will be sent to members of the consortium map committee for the construction of genetic maps. These maps will be circulated within the committee for study and comparison. The committee will meet for the final analysis of these maps, which will be used in the preparation of the consortium map. The consortium map and a report will be published in *Genomics*. It is this process that led to the CEPH consortium map of chromosome 10 presented in this issue of *Genomics*.

As each consortium map is published, the underlying genotypic data will be released to the scientific community. The consortium maps will provide the basis for choosing the primary mapped markers for which kits of probes will be distributed by CEPH to the scientific community in order to

enhance the use of the genetic map for localization of genes that determine disease and other genes of interest.

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³ A primary linkage map of a chromosome cannot be considered complete until it contains markers for, or quite close to, the telomeres.